

Effect of Dietary Patterns on Serum Homocysteine

Results of a Randomized, Controlled Feeding Study

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Background—Elevated blood levels of homocysteine are associated with an increased risk of atherosclerotic cardiovascular disease. Although numerous studies have assessed the impact of vitamin supplements on homocysteine, the effect of dietary patterns on homocysteine has not been well studied.

Methods and Results—During a 3-week run-in, 118 participants were fed a control diet, low in fruits, vegetables, and dairy products, with a fat content typical of US consumption. During an 8-week intervention phase, participants were then fed 1 of 3 randomly assigned diets: the control diet, a diet rich in fruits and vegetables but otherwise similar to control, or a combination diet rich in fruits, vegetables, and low-fat dairy products and reduced in saturated and total fat. Between the end of run-in and intervention periods, mean change in homocysteine was $+0.46 \mu\text{mol/L}$ in the control diet, $+0.21 \mu\text{mol/L}$ in the fruits and vegetables diet ($P=0.47$ compared with control), and $-0.34 \mu\text{mol/L}$ in the combination diet ($P=0.03$ compared with control, $P=0.12$ compared with the fruits and vegetables diet). In multivariable regression models, change in homocysteine was significantly and inversely associated with change in serum folate ($P=0.03$) but not with change in serum vitamin B₁₂ ($P=0.64$) or pyridoxal 5' phosphate, the coenzyme form of vitamin B₆ ($P=0.83$).

Conclusions—Modification of dietary patterns can have substantial effects on fasting levels of total serum homocysteine. These results provide additional insights into the mechanisms by which diet might influence the occurrence of atherosclerotic cardiovascular disease. (*Circulation*. 2000;102:852-857.)

Key Words: nutrition ■ risk factors ■ metabolism

A substantial body of evidence from observational epidemiological studies suggests that homocysteine is an independent risk factor for atherosclerotic cardiovascular disease (ASCVD). In 1969, McCully¹ first proposed the homocysteine theory of atherosclerosis. He postulated that homocysteine was responsible for the severe atherosclerosis observed in 2 distinct genetic conditions, that is, a rare disorder of vitamin B₁₂ metabolism and classic homocystinuria from homozygous cystathionine β -synthase deficiency. Since then, >30 observational studies have reported a direct relation between blood levels of homocysteine and ASCVD, including coronary heart disease, cerebrovascular disease, and peripheral vascular disease.² The relation is progressive and graded through ranges of homocysteine previously considered low or normal.³⁻⁷

Among the factors known to influence homocysteine metabolism are several nutrients, including folate, vitamin B₆, and vitamin B₁₂. Homocysteine can be remethylated to

methionine by methionine synthase in a reaction that requires methyltetrahydrofolate as a methyl donor and vitamin B₁₂ as an enzyme cofactor.⁸ Alternatively, homocysteine can be transsulfurated to cysteine in reactions that require vitamin B₆. In cross-sectional analyses from the Framingham Heart Study, elevated homocysteine was associated with low serum levels and low dietary intake of folate and vitamin B₆.⁹

Several trials have assessed the effects of folic acid, vitamin B₆, and vitamin B₁₂ supplements on homocysteine. In these trials, which typically enrolled persons with elevated homocysteine, high-dose folic acid supplements (often providing $\geq 1 \text{ mg/d}$) have reduced fasting levels of homocysteine.¹⁰ Vitamin B₆ supplements have had little impact on fasting levels of homocysteine but reduced the level of homocysteine after methionine loading.¹¹ Vitamin B₁₂ supplements have reduced homocysteine in individuals with vitamin B₁₂ deficiency but have minimal impact in healthy populations.^{12,13} However, because of differences in dose and

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bioavailability, the effects of vitamins derived from food should be different from that of high-dose vitamin supplements. For instance, it is well recognized that folic acid from vitamin supplements is better absorbed than dietary folate.¹⁴

Current dietary guidelines recommend increased consumption of fruits, vegetables, and low-fat dairy products (often milk, consumed with breakfast cereals).¹⁵ Unanticipated benefits of these diet recommendations may be an increase in folate, vitamin B₆, and vitamin B₁₂ intake and consequently a reduction in homocysteine, which could potentially lower the risk of ASCVD. Cross-sectional analyses from the Framingham Heart Study indicate that frequent consumption of certain foods, particularly, fruits, vegetables, and cereals, is correlated with low plasma levels of homocysteine,¹⁶ perhaps as a result of the high folate intake content of these foods. Nonetheless, inferences about causality must be made cautiously because of the potential for residual and uncontrolled confounding from other nutrients and nonnutritional factors.¹⁷ One controlled feeding study conducted in a metabolic ward suggested that folate-deficient diets may raise homocysteine, but the diets were unusual because of the artificially low intake of folate, just 25 and 90 $\mu\text{g}/\text{d}$.¹⁸

The objective of this study was to describe the effect of specific dietary patterns on fasting levels of total serum homocysteine in the setting of a randomized, controlled feeding study.

Methods

This research was an ancillary study in the Dietary Approaches to Stop Hypertension (DASH) trial, a multicenter trial designed to assess the effects of dietary patterns on blood pressure. This ancillary study, which was conducted at the DASH clinical center at Johns Hopkins, involved only the coauthors rather than the entire DASH collaborative group. Detailed descriptions of the design and methods of DASH¹⁹ and of its recruitment procedures²⁰ and main results²¹ have been published. A local institutional review board approved the trial protocol. Each participant provided written informed consent.

Participants

Trial participants were adults (age ≥ 22 years) who were not taking antihypertensive medication and who had an average systolic blood pressure < 160 mm Hg and average diastolic blood pressure of 80 to 95 mm Hg. Major exclusion criteria were poorly controlled diabetes; hyperlipidemia; cardiovascular event within 6 months; unwillingness to stop all vitamin and mineral supplements; use of medications that affect blood pressure; > 14 alcoholic drinks per week; and a glomerular filtration rate of < 50 mL/min (as estimated by the Cockcroft Gault formula). Participants were enrolled sequentially into groups. The first group began controlled feeding in September 1994. The last group ended feeding in April 1996, before routine fortification of food with folic acid.

Trial Conduct

After a screening period, eligible and interested participants began a 3-week run-in period in which they ate the control diet. During the third week, individuals were randomly assigned to 1 of 3 diets. For the next 8 weeks, participants ate their randomly assigned diets. During the last week of run-in and intervention, specimens of serum were obtained after overnight fasts. Each specimen was collected at room temperature, allowed to clot over 15 minutes, centrifuged at 2000g for 15 minutes at 4°C, and then placed in storage at -70°C until July 1996, when analyses were performed. Staff blinded to diet assignment collected all follow-up data.

TABLE 1. Nutrient Composition and Average Daily Servings of Food Groups for 2600-kcal Level of the Diets

	Diet		
	Control	Fruits and Vegetables	Combination
Macronutrient profile,* % kcal			
Fat	37	37	27
Saturated fat	14	13	7
Protein	14	15	18
Carbohydrate	50	52	59
Vitamin content, from database analyses*			
Folate, μg	207	390	428
Vitamin B ₆ , mg	1.8	3.1	2.7
Vitamin B ₁₂ , μg	3.7	3.8	4.9
Vitamin content, from chemical analyses†			
Folate, μg	168	314	418
Vitamin B ₁₂ , μg	4.7	4.6	6.5
Servings per day of food groups			
Fruits including juices	1.9	5.6	5.3
Vegetables	2.1	3.3	5.2
Dairy	0.5	0.3	3.2
Dry cereals	0.6	0.5	0.7
Nuts, seeds, and legumes	0	0.7	0.7
Beef, pork, and ham	1.8	2.1	0.6
Poultry	0.9	0.5	0.7
Fish	0.3	0.4	0.7

*From database analyses of DASH menus.

†From chemical analyses of composited meals. Each value corresponds to the predicted value for the 2600-kcal level from standard curves generated for each diet and nutrient.

Dietary Patterns

The control diet was relatively low in fruits, vegetables, and dairy products, with a fat content typical of US consumption. A second diet was rich in fruits and vegetables but otherwise similar to the control diet. The combination diet emphasized fruits, vegetables, and low-fat dairy products. It included whole grains, poultry, fish, and nuts, and was reduced in fat, red meat, sweets, and sugar-containing beverages.²²

For the 2600-kcal level of the 3 diets, Table 1 displays the macronutrient profile and the content of folate, vitamin B₆, and vitamin B₁₂ as estimated from database analyses of the menus using Moore's Extended Nutrient System (MENUS, Pennington Biomedical Research Center, Baton Rouge, La); the folate and vitamin B₁₂ content as estimated from chemical analyses of composited meals; and the average number of servings per day of selected food groups. For the nutrient estimates derived from meal composites, a full week cycle of meals at each of 4 calorie levels was composited, stored, and then analyzed for folate and vitamin B₁₂. For each diet and nutrient, a standard curve was generated from which the predicted value at 2600 kcal was estimated.

Controlled Feeding

On each weekday of the 11-week feeding period, participants ate either lunch or dinner at the clinical center. After completing the on-site meal, participants received coolers that contained the other meals to be consumed off-site. On Fridays, they also received their weekend meals, all consumed off-site. Self-report of perfect adher-

TABLE 2. Characteristics of Trial Participants

Characteristic*	Diet			
	Control (n=39)	Fruits and Vegetables (n=41)	Combination (n=38)	All (n=118)
Age, y	50 (40, 58)	49 (45, 56)	48.5 (39, 54)	49 (40, 56)
% Women	51.3%	43.9%	50%	48.3%
% Minority	66.7%	63.4%	71.1%	67.0%
% Current smokers	12.8%	9.8%	13.2%	11.9%
Systolic blood pressure, mm Hg	133.1 (126.0, 144.3)	136.1 (128.9, 143.3)	130.6 (125.4, 139.7)	133.2 (127.0, 141.3)
Diastolic blood pressure, mm Hg	87.0 (82.7, 88.4)	85.9 (82.4, 89.4)	83.5 (81.1, 87.0)	85.1 (82.3, 88.4)
Creatinine, mg/dL	1.0 (0.9, 1.1)	1.1 (1.0, 1.2)	1.0 (0.9, 1.1)	1.0 (0.9, 1.2)
Prestudy				
Vitamin use†	17.9%	12.2%	23.7%	17.8%
Folate intake‡	314 (228, 406)	319 (226, 438)	304 (254, 446)	313 (251, 446)
Vitamin B ₆ intake‡	2.0 (1.4, 2.6)	1.8 (1.4, 2.5)	1.8 (1.5, 2.5)	1.8 (1.4, 2.5)
Run-in serum level of				
Homocysteine, μ mol/L	8.93 (6.78, 11.24)	9.29 (7.89, 12.37)	9.34 (6.80, 12.09)	9.23 (7.32, 11.95)
Folate, μ g/L	4.51 (3.72, 6.20)	5.32 (3.87, 7.37)	5.72 (4.00, 6.71)	5.09 (3.87, 6.81)
PLP, nmol/L	41.6 (32.0, 60.0)	41.5 (35.2, 63.9)	42.2 (32.4, 52.4)	41.5 (33.0, 59.6)
Vitamin B ₁₂ , ng/L	392 (350, 453)	436 (361, 508)	430 (343, 520)	423 (350, 508)

*Continuous data are presented as median (first quartile, third quartile).

†Prestudy use of multivitamins or B-complex vitamin supplements.

‡From a food frequency questionnaire completed before run-in (control, n=34; fruits and vegetables, n=36; combination, n=37; all, n=107).

ence (no nonstudy foods consumed and all study foods eaten) occurred in 96%, 96%, and 94% of person-days in those assigned to the control, fruits and vegetables, and combination diets.

A 7-day menu cycle with 21 meals at 4 calorie levels (1600, 2100, 2600, and 3100 kcal) was developed for each diet. Food was prepared in metabolic kitchens, primarily at the Beltsville Human Nutrition Research Center of the US Department of Agriculture, and then served at the Hopkins clinical center. Weight was measured each weekday and was kept stable by adjusting calorie intake.

Laboratory Assays

Total serum homocysteine (free and protein bound) was determined by high-performance liquid chromatography according to the method of Araki and Sako²³; the between-run coefficient of variation (CV) for this assay was 8%. Serum folate and vitamin B₁₂ were measured by radioimmune assay with the use of a kit from Bio-Rad; the between-run CVs for these assays were 10% and 7%, respectively. Folate in aliquots of composited meals was measured with the use of a microbial assay after conjugase treatment.²⁴ Pyridoxal-5'-phosphate (PLP), the coenzyme form of vitamin B₆, was measured by the tyrosine decarboxylase method, based on principles described by Shin-Buehring et al²⁵; the between-run CV for this assay was 16%.

Statistical Considerations

Change in fasting levels of total serum homocysteine between the end of run-in and intervention periods was the primary outcome variable. The target sample size of 114 at the Hopkins clinical center was estimated to provide 80% power to detect a mean between-diet difference of 2 μ mol/L in homocysteine. Analyses were performed on an intention-to-treat basis. For each outcome variable, run-in values and changes from run-in tended to be normally distributed; however, several outliers were present. To minimize the potential influence of these outliers, we displayed baseline data as medians with interquartile ranges and used robust regression analyses to test for differences between randomized groups. In each regression model, the dependent variable was change from end of run-in to end

of intervention. Covariates in each model were the run-in level of the dependent variable as well as 2 indicator variables corresponding to diet assignment. Trends across the 3 diets (control, fruits and vegetables, and combination) were tested in separate models by entering an ordinal variable (0, 1, 2) corresponding to these 3 diets. To explore the potential influence of nutrients that affect homocysteine metabolism, we calculated Spearman correlations between homocysteine and levels of folate, PLP, and vitamin B₁₂ at end of run-in and intervention, and between changes in homocysteine and changes in folate, PLP, and vitamin B₁₂. To assess the independent association of change in homocysteine with changes in folate, PLP, and vitamin B₁₂, we simultaneously entered changes in folate, PLP, and vitamin B₁₂ in a multivariable regression model. Analyses were performed with the use of Stata 6.0 and SAS 6.12 software.

Results

Of the 135 who started run-in, 124 (92%) were randomized. Paired specimens of serum, collected at the end of run-in and intervention periods, were available in 118 persons (95% of randomized participants), all of whom completed the 11 weeks of controlled feeding. As displayed in Table 2, participants tended to be middle-aged (median age of 49 years, range 23 to 76 years). Approximately half were women, and two thirds came from a minority background, predominantly black. Before feeding, median folate intake was 313 μ g/d, and few individuals (<20%) were users of multivitamins or B-complex vitamin supplements.

Effects of Dietary Patterns on Serum Folate, PLP, and Vitamin B₁₂

Figures 1, 2, and 3 display mean (95% CI) changes in serum folate, PLP, and vitamin B₁₂ between the end of run-in and intervention after adjustment for run-in levels. Mean change in serum folate was -0.80μ g/L in the control group, $+0.10$

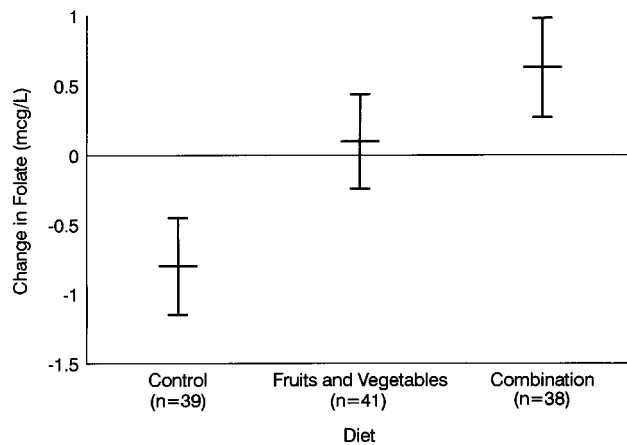


Figure 1. Mean (95% CI) change in serum folate ($\mu\text{g/L}$) from end of run-in to end of intervention.

$\mu\text{g/L}$ in the fruits and vegetables group ($P<0.001$ compared with control), and $+0.63 \mu\text{g/L}$ in the combination group ($P<0.001$ compared with control, $P=0.04$ compared with fruits and vegetables). For serum PLP, mean change was -2.8 nmol/L in the control group, $+8.4 \text{ nmol/L}$ in the fruits and vegetables group ($P<0.001$ compared with control), and $+4.3 \text{ nmol/L}$ in the combination group ($P=0.03$ compared with control, $P=0.19$ compared with fruits and vegetables). Mean change in vitamin B_{12} was -16 ng/L in the control group, -13 ng/L in the fruits and vegetables group ($P=0.81$ compared with control), and 8.0 ng/L in the combination group ($P=0.08$ compared with control, $P=0.12$ compared with fruits and vegetables).

Homocysteine

As displayed in Figure 4, mean within-group change in homocysteine, after adjustment for run-in level, was $+0.46 \mu\text{mol/L}$ (95% CI $-0.04, +0.96$) in the control group, $+0.21 \mu\text{mol/L}$ (95% CI $-0.27, +0.69$) in the fruits and vegetables group, and $-0.34 \mu\text{mol/L}$ (95% CI $-0.84, +0.16$) in combination group. Between-diet differences were $-0.8 \mu\text{mol/L}$ (95% CI $-1.51, -0.1$; $P=0.03$) comparing control and combination groups, $-0.25 \mu\text{mol/L}$ (95% CI $-0.94, 0.44$; $P=0.47$) comparing the control group and the

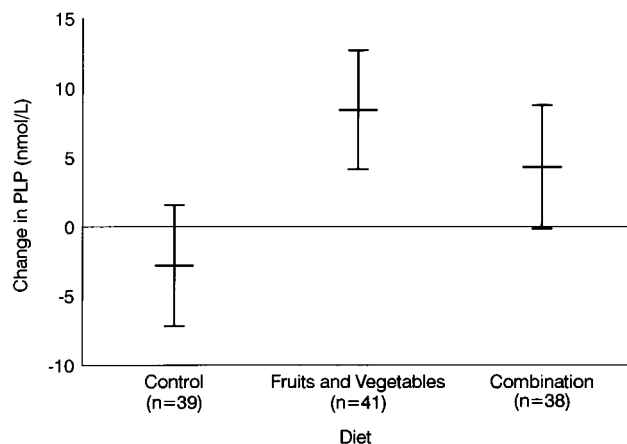


Figure 2. Mean (95% CI) change in serum PLP (nmol/L) from end of run-in to end of intervention.

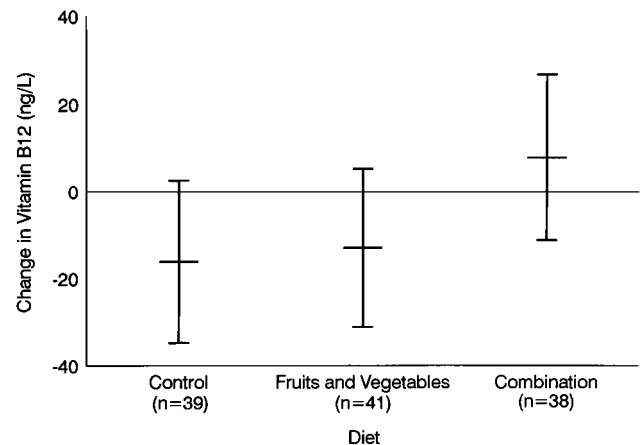


Figure 3. Mean (95% CI) change in serum vitamin B_{12} (ng/L) from end of run-in to end of intervention.

fruits and vegetables group, and $-0.55 \mu\text{mol/L}$ (95% CI $-1.24, 0.15$; $P=0.12$) comparing the fruits and vegetables group and the combination group. Across the 3 diets, there was a progressive reduction in homocysteine (P for trend $=0.02$).

Correlates of Homocysteine

At the end of run-in, serum homocysteine was significantly and inversely correlated with serum folate ($r=-0.54$, $P=0.0001$) and vitamin B_{12} ($r=-0.34$, $P=0.0002$) but not PLP ($r=-0.06$, $P=0.54$). An identical pattern of findings was present in analyses correlating end-of-intervention homocysteine with end-of-intervention serum nutrients. However, in analyses correlating change in homocysteine with changes in nutrients, change in homocysteine was associated with change in serum folate ($r=-0.28$, $P=0.002$) but not with change in PLP ($r=-0.02$, $P=0.79$) or vitamin B_{12} ($r=-0.12$, $P=0.21$). In regression analyses that simultaneously adjusted for changes in serum folate, PLP, and vitamin B_{12} and for run-in level of homocysteine, change in serum homocysteine was only associated with change in folate (Table 3).

Discussion

This trial demonstrated that modification of dietary patterns can have substantial effects on fasting levels of serum

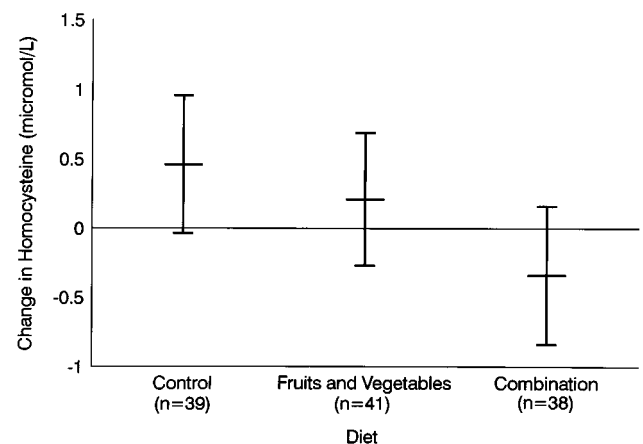


Figure 4. Mean (95% CI) change in serum homocysteine ($\mu\text{mol/L}$) from end of run-in to end of intervention.

TABLE 3. Relation Between Change in Homocysteine and Changes in Serum Nutrients (Folate, PLP, and Vitamin B₁₂) Results From a Multivariable Robust Regression Model Adjusting for Run-In Level of Homocysteine

Change in Serum	Coefficient*	95% CI	P
Folate, $\mu\text{g/L}$	-0.23	-0.43, -0.03	0.03
PLP, nmol/L	0.002	-0.015, 0.012	0.83
Vitamin B ₁₂ , ng/L	0.001	-0.005, 0.003	0.864

*Change in serum concentration of homocysteine ($\mu\text{mol/L}$) per unit change in serum concentration of nutrient.

homocysteine. Specifically, in a population of individuals with high normal blood pressure or stage 1 hypertension, a control diet that was relatively low in fruits, vegetables, and dairy products with a fat content typical of US consumption raised homocysteine. In contrast, the DASH combination diet that was diet rich in fruits, vegetables, and low-fat dairy products and reduced in saturated and total fat lowered homocysteine. A diet rich in fruits and vegetables but otherwise similar to the control diet had an intermediate effect. Recent data suggest that a 3- to 4- $\mu\text{mol/L}$ reduction in homocysteine should lower vascular disease risk by one third.² If homocysteine proves to be an independent ASCVD risk factor, then the observed 0.8- $\mu\text{mol/L}$ difference in homocysteine between control and combination diets should lower ASCVD risk by 7% to 9% among persons consuming a typical American diet who subsequently adopt the DASH combination diet.

Among the strengths of this study are high internal and external validity. Follow-up data were collected in 95% of randomized participants. Furthermore, adherence was excellent as indicated by self-reports of food consumption, by changes in serum levels of nutrients (documented in this ancillary study), and by changes in the urinary excretion of electrolytes (documented in the overall trial²¹). We attribute much of our successful follow-up and adherence to the 3-week run-in period that preceded randomization. Also, the study population was demographically heterogeneous. Nearly half of trial participants were women, two thirds were from a minority background, and the age range was broad. In addition, the median level of fasting homocysteine in this trial was similar to corresponding data from a large national survey.²⁶ Finally, the combination diet was broadly consistent with national dietary recommendations.¹⁵

Potential limitations of this trial include the duration of feeding (11 weeks), the relatively small sample size (118 persons allocated across 3 groups), and the potential influence of prestudy diets of participants on trial results. On the basis of data from a food frequency questionnaire, prestudy folate intake was $>300 \mu\text{g/d}$. In the control group, which received a diet with $<250 \mu\text{g/d}$ folate, homocysteine rose between run-in and intervention despite the fact that this group remained on the same diet. One explanation for this finding pertains to the timing of specimen collection; baseline specimens were drawn after just 3 weeks of run-in feeding, a point at which vitamin stores and homocysteine may still have reflected, to some extent, the prestudy dietary intake of participants. The observation that serum folate fell between

run-in and intervention in the control group supports the notion that participants had not reached a steady state, at least with respect to folate balance by the end of run-in.

Several aspects of the diets might explain the observed changes in homocysteine, including the gradient across diets. First, dietary folate increased progressively across the diets, with the lowest intake in the control diet and the highest in the combination diet. In exploratory analyses, change in serum folate was significantly and independently correlated with change in homocysteine; no other nutrient was correlated with homocysteine change. Folate-rich foods that might have contributed to the reductions in homocysteine observed in the combination diet include fruits, juices, vegetables, and perhaps dairy products.

Vitamin B₁₂ may also have had a beneficial effect on fasting levels of homocysteine. The combination diet provided more vitamin B₁₂ than either the control diet or the fruits and vegetables diet. Serum vitamin B₁₂ was significantly correlated with homocysteine at the end of both run-in and intervention. However, change in homocysteine was not significantly associated with change in serum vitamin B₁₂. The absence of a relation between change in serum vitamin B₁₂ and change in homocysteine may have resulted from the fact that serum vitamin B₁₂ levels changed minimally over the 8-week intervention period.

Overall, our data suggest that dietary folate intake had a major influence on fasting levels of homocysteine and that vitamin B₁₂ may also have had an effect. Such findings are consistent with the known metabolism of homocysteine,⁸ with cross-sectional analyses of observational studies,⁹ and with clinical trials of vitamin supplements.¹⁰⁻¹² Nonetheless, the trial was designed to test the effects of whole dietary patterns rather than the effects of individual nutrients. The associations of folate and homocysteine, albeit robust, could be distorted (either artificially increased or diminished) from the effects of correlated nutrients, such as vitamin B₁₂, which also affect homocysteine levels. Furthermore, in addition to the well-known determinants of fasting homocysteine, the diets differed in several other aspects, for example, protein intake, which might influence homocysteine metabolism.²⁷

Results of this trial may help to explain the beneficial effects of certain dietary patterns, such as vegetarian diets, which are associated with a reduced risk of ischemic heart disease and stroke.^{28,29} Although the nutrients responsible for such effects are uncertain, attention has focused on reduced consumption of certain nutrients, such as saturated fat, and increased consumption of potassium, fiber, and naturally occurring antioxidants, such as β -carotene and lycopene. Results from this study suggest that another mechanism, namely, a reduction in homocysteine, may in part be responsible for the beneficial effects of these diets, many of which are excellent sources of folate.

In summary, modification of dietary patterns can have substantial effects on fasting levels of total serum homocysteine. These results provide additional insights into the mechanisms by which diet might influence the occurrence of atherosclerotic cardiovascular disease.

Acknowledgments

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